

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
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 Office  
 Box PCT  
 Washington, D.C.20231  
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

<b>Date of mailing (day/month/year)</b> 08 February 2000 (08.02.00)	
<b>International application No.</b> PCT/NL99/00316	<b>Applicant's or agent's file reference</b> WO 800101-AI
<b>International filing date (day/month/year)</b> 20 May 1999 (20.05.99)	<b>Priority date (day/month/year)</b> 20 May 1998 (20.05.98)
<b>Applicant</b> VAN ASBECK, Bernt, Sweder et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

16 December 1999 (16.12.99)

☐ in a notice effecting later election filed with the International Bureau on:
2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No.: (41-22) 740.14.35	Authorized officer  Claudio Borton  Telephone No.: (41-22) 338.83.38
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# PCT

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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**PCT/NL 9 9 / 0 0 3 1 6**

International Application No. \_\_\_\_\_

**20 MAY 1999**

(20.05.99)

International Filing Date

BUREAU VOOR DE INDUSTRIËLE EIGENDOM  
P.C.T. INTERNATIONAL APPLICATION

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference  
(if desired) (12 characters maximum) WO 800101-AI

**Box No. I TITLE OF INVENTION**

Use of a nucleic acid-comprising chemotherapeutic agent, and a pharmaceutical composition

**Box No. II APPLICANT**

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

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Universiteitsweg 100  
NL-3508 TA UTRECHT  
the Netherlands

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (that is, country) of nationality:  
NL

State (that is, country) of residence  
NL

This person is applicant  
for the purposes of:

☐ all designated  
States

☒ all designated States except  
the United States of America

☐ the United States  
of America only

☐ the States indicated in  
the Supplemental Box

**Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

VAN ASBECK, Bernt Sweder  
Verheulfaan 19  
NL-3971 RD DRIEBERGEN  
the Netherlands

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box  
is marked, do not fill in below.)

State (that is, country) of nationality:  
NL

State (that is, country) of residence:  
NL

This person is applicant  
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**Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The person identified below is hereby/has been appointed to act on behalf  
of the applicant(s) before the competent International Authorities as:

☒ agent

☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

ALTENBURG, Bernardus Stephanus Franciscus et al.  
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Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS	
<i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) <b>MARX, Johannes Josephus Maria</b> <b>Johan Buziaulaan 41</b> <b>NL-3584 ZT UTRECHT</b> <b>the Netherlands</b>	This person is: <input type="checkbox"/> applicant only <input checked="" type="checkbox"/> applicant and inventor <input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)
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This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: <input type="checkbox"/> applicant only <input type="checkbox"/> applicant and inventor <input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)
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Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: <input type="checkbox"/> applicant only <input type="checkbox"/> applicant and inventor <input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)
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**Box No.V DESIGNATION STATES**

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

**Regional Patent**

- ☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
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- ☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line) .....

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Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
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**Precautionary Designation Statement:** In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

<b>Box No. VI PRIORITY CLAIM</b>		<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.		
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: regional Office	international application: receiving Office
item (1) (20.05.98) 20 May 1998	1009226	NL		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): 1

\* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

**Box No. VII INTERNATIONAL SEARCHING AUTHORITY**

<b>Choice of International Searching Authority (ISA)</b> (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):	<b>Request to use results of earlier search; reference to that search</b> (if an earlier search has been carried out by or requested from the International Searching Authority):		
ISA /	Date (day/month/year)	Number	Country (or regional Office)

**Box No. VIII CHECK LIST: LANGUAGE OF FILING**


This international application contains the following number of sheets: request : 4 description (excluding sequence listing part) : 5 claims : 1 abstract : 1 drawings : 1 sequence listing part of description : Total number of sheets : 12	This international application is accompanied by the item(s) marked below: 1. <input checked="" type="checkbox"/> fee calculation sheet 2. <input type="checkbox"/> separate signed power of attorney 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: 4. <input type="checkbox"/> statement explaining lack of signature 5. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s): 6. <input type="checkbox"/> translation of international application into (language): 7. <input type="checkbox"/> separate indications concerning deposited microorganism or other biological material 8. <input type="checkbox"/> nucleotide and/or amino acid sequence listing in computer readable form 9. <input checked="" type="checkbox"/> other (specify): Copy of Search Report
--	---

Figure of the drawings which should accompany the abstract:	Language of filing of the international application: Dutch
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**Box No. IX SIGNATURE OF APPLICANT OR AGENT**

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Amsterdam, 20 May 1999



ALTENBURG, Bernardus Stephanus Franciscus et al.

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1. Date of actual receipt of the purported international application: 20 MAY 1999 (20.05.99)		
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 1(2):		
5. International Searching Authority (if two or more are competent): ISA /	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.	

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Date of receipt of the record copy by the International Bureau:	23 JUNE 1999 (23.06.99)

WO 800101-A1/ho

Toepassing van een nucleïnezuur-bindend chemotherapeutisch agens, en een farmaceutisch preparaat

De onderhavige uitvinding heeft betrekking op een toepassing van een nucleïnezuur-bindend chemotherapeutisch agens, waarbij het nucleïnezuur-bindende chemotherapeutische agens een metaalion kan complexeren onder oplevering van een  
5 complex dat de vorming van hydroxylradicalen uit waterstofperoxide bevordert.

Een dergelijk nucleïnezuur-bindend chemotherapeutisch agens is in het vak bekend. Zo wordt voor de behandeling van bepaalde neoplastische weefsels (tumoren) bleomycine  
10 gebruikt. Bleomycine kan tweewaardig ijzer binden en het ijzerion behoudt daarbij het vermogen om de vorming van hydroxylradicalen uit waterstofperoxide te bevorderen.

De onderhavige uitvinding beoogt een nieuwe toepassing te verschaffen van een zoals hierboven gedefinieerd nucleïnezuur-bindend chemotherapeutisch agens.  
15

Volgens de onderhavige uitvinding kan het nucleïnezuur-bindende chemotherapeutische agens worden gebruikt voor de bereiding van een virion-aantal reducerend middel.

Verrassenderwijs is gebleken dat de virusrePLICatie  
20 onder gebruikmaking van het hierboven gedefinieerde nucleïnezuur-bindende chemotherapeutische agens kan worden geremd, zonder dat de gastheercel daar zichtbaar onder lijdt. Zonder aan enige theorie gebonden te zijn, meent aanvraagster dat de remming specifiek is doordat met name in met een virus geïnfecteerde cellen de vorming van hydroxylradicalen uit waterstofperoxide wordt bevorderd.  
25

Volgens een voorkeursuitvoering is het nucleïnezuur-bindende chemotherapeutische agens gekozen uit de groep bestaande uit bleomycine, adriamycine, en derivaten daarvan.

30 Deze verbindingen beschikken over uitstekende een metaalion complexerende eigenschappen. In het bijzonder zijn zij in staat om ijzerionen in het lichaam van een patiënt te binden. Hierdoor is het gevormde ijzer-bleomycine complex in staat in een cel de vorming van hydroxylradicalen uit waterstofperoxide te bevorderen.  
35

Bij voorkeur wordt het nucleïnezuur-bindende chemotherapeutische agens gebruikt voor de bereiding van een RNA virus-replicatie remmend middel, in het bijzonder wordt het nucleïnezuur-bindende chemotherapeutische agens gebruikt voor  
5 de bereiding van een HIV-replicatie-remmend middel.

Carter, B.J. et al. (Proc. Natl. Acad. Sci. USA, deel 87, blz. 9373-9377 (1990)) beschrijven de invloed van Fe(II)-bleomycine complex op mRNA dat codeert voor reverse transcriptase van HIV-1. Het beschreven experiment is uitge-  
10 voerd in een cel-vrij systeem. Er is geen indicatie dat preferentieel in geïnfecteerde cellen de vorming van hydroxylradicalen uit waterstofperoxide wordt bevorderd.

De uitvinding heeft verder betrekking op een farmaceutisch combinatie-preparaat dat een nucleïnezuur-bindend  
15 chemotherapeutisch agens dat een ermee gecomplexeerd metaalion omvat, welk complex de vorming van hydroxylradicalen uit waterstofperoxide kan bevorderen, te zamen met een farmaceutisch aanvaardbare drager of vulmiddel omvat, alsmede een ijzer-chelaterende verbinding welke ijzer in een vorm bindt  
20 waarin het de vorming van hydroxylradicalen uit waterstofperoxide niet kan bevorderen.

Een dergelijke ijzerchelatorcombinatie, dat desgewenst 2 afzonderlijke farmaceutische preparaten omvat met elk een van de respectievelijke actieve bestanddelen,  
25 maakt het mogelijk om de vorming van de hydroxylradicalen specifiek te localiseren. Door gebruik van een ijzer-chelaterende verbinding die cellen niet binnen kan dringen, kan preferentieel de vorming van ijzer-bleomycine complex buiten de cellen worden beperkt, en daarmee ook de schade die een  
30 dergelijk complex aanricht worden verminderd. Gebruik van een ijzer-chelaterende verbinding die de cellen wel binnen kan dringen beperkt de hoeveelheid ijzerionen die de vorming van hydroxylradicalen beperkt. Hierdoor kan ten minste een deel van het activatieproces van de transcriptiefactor Nuclear  
35 Factor kappa B (NFκB), dat de virus replicatie kan stimuleren, in het cytoplasma worden beperkt. Wel dient ervoor te worden gezorgd dat er ijzer voor bleomycine beschikbaar is. Een arts kan dit bereiken door het kiezen van geschikte doses van de beide actieve bestanddelen afhankelijk van het

lichaamsgewicht van de te behandelen persoon, en diens beschikbare hoeveelheid ijzer. Volgens een gunstige uitvoeringsvorm wordt daarbij een ijzer-chelaterende verbinding gekozen met een ijzer-chelaterend vermogen dat bij voorkeur ten minste 3 keer lager is, met meer voorkeur ten minste 10 keer lager is dan dat van het nucleïnezuur-bindende chemotherapeutische agens.

Aldus kan worden bevorderd dat er, door de grotere affiniteit van bleomycine voor ijzer, actief ijzer-bleomycine complex in geïnfecteerde cellen aanwezig is en dat, in het bijzonder, de extracellulaire schadelijke effecten van bleomycine complex worden beperkt.

Aanvraagster houdt rekening met de mogelijkheid dat het gebruik van een ijzer-chelaterende verbinding zoals hierboven is gedefinieerd ook toepassing kan vinden bij het beperken van ongewenste schade die optreedt bij behandeling van neoplastische weefsels met een nucleïnezuur-bindende chemotherapeutische agens zoals bleomycine.

De onderhavige uitvinding zal thans worden toegelicht aan de hand van het navolgende voorbeeld en onder verwijzing naar de tekening waarin

fig. 1 een grafiek voorstelt waarin het effect van bleomycine op de HIV-1 replicatie in macrofagen is uitgezet;

fig. 2 een grafiek voorstelt waarin de cytotoxiciteit van bleomycine voor macrofagen is uitgezet;

fig. 3 een grafiek voorstelt waarin het effect van bleomycine op de HIV-1 replicatie in lymfocyten is uitgezet;

fig. 4 een grafiek voorstelt waarin het effect is uitgezet van de concentratie bleomycine op de lymfocytoprolieratie.

#### Voorbeeld

Macrofagen en lymfocyten ( $10^6$  cellen/ml) werden gedurende 2 uur geïnfecteerd met HIV-1<sub>Ba-L</sub>. De verhouding HIV-deeltjes/aantal cellen was 0,005 voor macrofagen en 0,001 voor lymfocyten. De geïnfecteerde cellen werden vervolgens twee keer gewassen teneinde overmaat virus te verwijderen. De cellen werden gedurende vijf dagen in RPMI 1640 medium (aangevuld met 10% foetaal kalfsserum, 10 U/ml IL-2, 10 µg/ml gentamycine, en 0,5 µg/ml ciprofloxamine) geïncubeerd met 3 ij-



zerchelatoren, te weten Deferoxamine (DI; Novartis Pharma, Arnhem Nederland), Deferiprone (L1; Duchefa Farma B.V., Haarlem, Nederland) of Bleomycine (BLM; H. Lundbeck A/S, Kopenhagen, Denemarken). Virus in kweeksupernatant werd

5 geïnactiveerd met Empigen (Calbiochem-Novabiochem Co., La Jolla, Californië, Verenigde Staten van Amerika) in een eindconcentratie van 0,05% en daaropvolgend verwarmen gedurende 30 minuten bij 56°C. De p24-concentratie werd gemeten met behulp van een ELISA als maat voor de replicatie van HIV-1

10 (Moore, J.P. et al., Science 250, blz. 1139-1142 (1990)). Cytotoxiciteit metingen werden uitgevoerd onder gebruikmaking van een fluorescence-activated cell sorter onder gebruikmaking van kleuring met propidiumjodide en DiOC5 (3,3'-diapentiloxacarboxyaminejodide). De proliferatie van lymfocyten

15 werd gemeten door opname van <sup>3</sup>H-thymidine. Uit figuur 1 en figuur 2 blijkt de dosis-afhankelijke reductie van de HIV-1 replicatie. De beperkte cytotoxiciteit van bleomycine voor macrofagen blijkt uit figuur 3. Het geringe effect van bleomycine op de lymfocytoproliferatie blijkt uit figuur 4. Uit

20 het feit dat de cellulaire proliferatie met bleomycine over een groot concentratiebereik intact blijft, in tegenstelling tot DF en L1 die wel de celproliferatie remmen (resultaten niet afgebeeld. L1 remt de proliferatie in hoofdzaak volledig met 10 µM), volgt dat er sprake is van een ander, niet op

25 proliferatieremming gebaseerd mechanisme. Tevens is de door BLM-geïnduceerde reductie van HIV replicatie niet een gevolg van cytotoxische effecten van BLM.

In een poging meer te weten te komen op welk niveau het nucleïnezuur-bindende chemotherapeutische agens aangrijpt

30 voor het reduceren van het virionaantal in een geïnfecteerde cel, is gekeken naar transcriptiefactoren die zich op HIV-LTR (HIV-Long Terminal Repeat) bevinden, waarvan NFκB een belangrijke rol speelt bij virale transcriptie. NFκB is nodig voor de initiatie van de transcriptie van pro-viraal DNA aanwezig

35 in het gastheergenoom. Uit EMSE-analyse (Electrophoretic Mobility Shift Assay) van NFκB in kernextracten bleek dat bleomycine geen effect had op NFκB-activatie, wat suggereert dat HIV-remming door bleomycine langs een andere route gaat dan transcriptieremming. Het feit dat NFκB uit kernextracten be-

reid van met 20 ng/ml phorbolmyristaatacetaat (PMA)-gestimuleerde Jurkat cellen niet door BLM werd geremd (concentraties tot 3 µg/ml), suggereert dat de remming van HIV-1 door BLM anders gebeurt dan voorgesteld voor conventionele ijzerchela-  
 5 toren zoals DF (Sappey et al. Aids Res. Hum. Retroviruses 11, blz. 1049-1061 (1995)).

Om te onderzoeken of bleomycine in een eerder stadium, dat wil zeggen vóór integratie in het genoom, aangrijpt, werden de viraal DNA-beschadigende eigenschappen van BLM in  
 10 met HIV-1 geïnfecteerde perifeer bloed lymfocyten (PBL) onderzocht. Daartoe werden de producten van reverse transcriptie waaronder de eerste minusstreng strong stop DNA geamplificeerd onder gebruikmaking van de R/U5 primers: sense 5'-GGCTAACTAGGGAACCCACTG-3' en antisense 5'-CTGCTAGAGATTTTCCA-  
 15 CACTGAC-3' (aan 5' uiteinde biotinyleerd), wat resulteerde in een fragment van 140 bp. Voor het kwantificeren van dit fragment werd een met digoxigenine gelabelde probe 5'-TGTGTGCCCCGTCTGTTGTGTG-3' gebruikt. Kwantificering geschiedde met behulp van een DIG-detectie ELISA (Boehringer-Mannheim,  
 20 Mannheim, Duitsland). Strong stop DNA, gevormd in met HIV geïnfecteerde perifeer bloedlymfocyten (PBL), was na incubatie met BLM vrijwel afwezig. Dit zou kunnen betekenen dat hetzij het reverse transcriptase enzym wordt geremd, of dat de DNA-producten van reverse transcriptase direct door BLM  
 25 worden beschadigd.

Op basis van de uitgevoerde experimenten wordt gedacht dat bleomycine viraal DNA en/of RNA in het cytoplasma beschadigt. De ter controle gemeten GAPDH-DNA-concentratie (GAPDH is glyceraldehyde-3-fostaat dehydrogenase) in de cel  
 30 blijft in hoofdzaak constant, wat het idee ondersteunt dat het gastheer DNA vrij goed tegen BLM is beschermd, en dat BLM bij voorkeur DNA/RNA in het cytosol aantast, in dit geval viraal DNA/RNA. Dit zou ook een verklaring kunnen geven waarom in het hierboven als eerste beschreven experiment de p24-  
 35 waarden na het incuberen van cellen met BLM niet volledig zijn teruggebracht. Immers, aangezien de cellen gedurende 2 uur in afwezigheid van BLM worden geïncubeerd, zal er ongetwijfeld enige pro-virale integratie in het gastheer genoom zijn opgetreden.

CONCLUSIES

1. Toepassing van een nucleïnezuur-bindend chemotherapeutisch agens voor de bereiding van een virion-aantal reducerend middel, waarbij het nucleïnezuur-bindend chemotherapeutisch agens een metaalion kan complexeren onder oplevering  
5 van een complex dat de vorming van hydroxylradicalen uit waterstofperoxide bevordert.

2. Toepassing volgens conclusie 1, met het kenmerk, dat het nucleïnezuur-bindend chemotherapeutisch agens is gekozen uit de groep bestaande uit bleomycine, adriamycine, en  
10 derivaten daarvan.

3. Toepassing volgens conclusie 1 of 2, met het kenmerk, dat het nucleïnezuur-bindend chemotherapeutisch agens wordt gebruikt voor de bereiding van een RNA virus-aantal reducerend middel.

15 4. Toepassing volgens conclusie 3, met het kenmerk, dat het nucleïnezuur-bindend chemotherapeutisch agens wordt gebruikt voor de bereiding van een HIV-replicatie-remmend middel.

5. Farmaceutisch combinatie-preparaat dat een  
20 nucleïnezuur-bindend chemotherapeutisch agens dat een ermee gecomplexeerd metaalion omvat, welk complex de vorming van hydroxylradicalen uit waterstofperoxide kan bevorderen, te zamen met een farmaceutisch aanvaardbare drager of vulmiddel omvat, alsmede een ijzer-chelaterende verbinding welke ijzer  
25 in een vorm bindt waarin het de vorming van hydroxylradicalen uit waterstofperoxide niet kan bevorderen.

6. Farmaceutisch combinatie-preparaat volgens conclusie 5, met het kenmerk, dat de ijzer-chelaterende verbinding een ijzer-chelaterend vermogen heeft dat bij voorkeur  
30 ten minste 3 keer lager is, met meer voorkeur ten minste 10 keer lager is dan dat van het nucleïnezuur-bindende chemotherapeutische agens.

UITTREKSEL

De uitvinding heeft betrekking op het toepassen van een nucleïnezuur-bindend chemotherapeutisch agens, zoals bleomycine, waarbij het nucleïnezuur-bindend chemotherapeutische agens een metaalion kan complexeren onder oplevering van  
5 een complex dat de vorming van hydroxylradicalen uit waterstofperoxide bevordert. Volgens de onderhavige uitvinding wordt het agens gebruikt voor de bereiding van een virion-aantal reducerend middel. De uitvinding heeft tevens betrekking op een farmaceutisch preparaat.

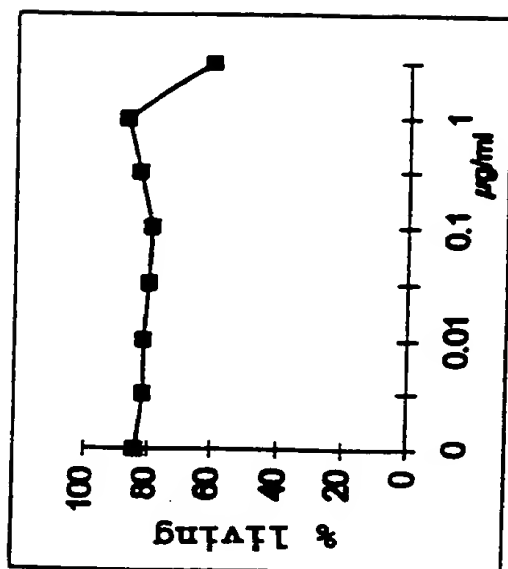


Fig. 2

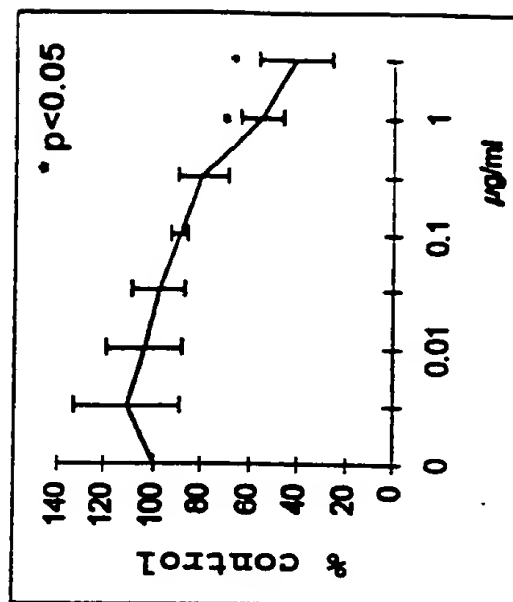


Fig. 4

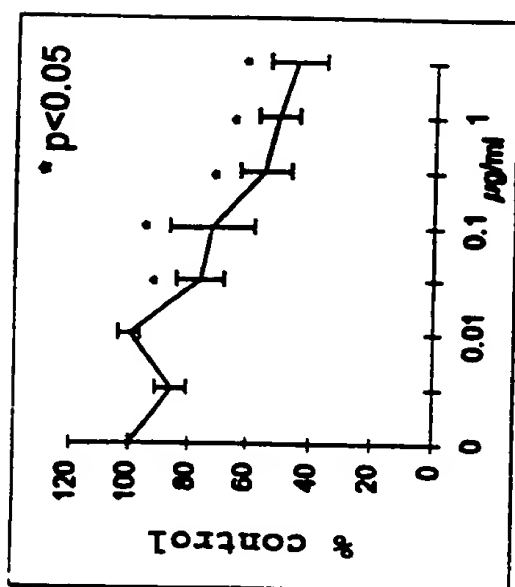


Fig. 1

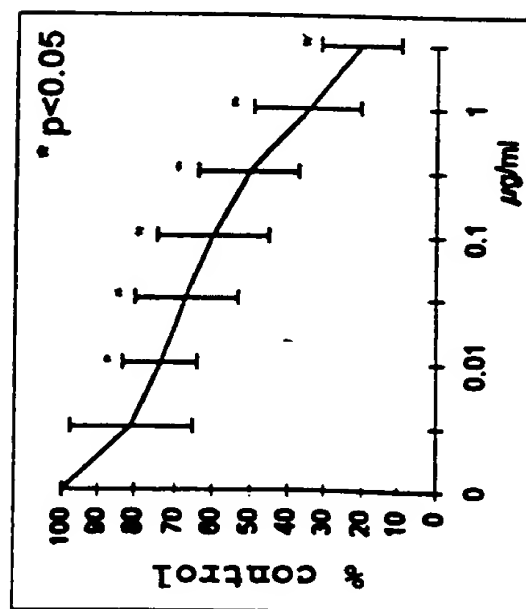


Fig. 3

# PATENT COOPERATION TREATY

# PCT

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

16

Applicant's or agent's file reference <b>WO 800101-AI</b>	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/NL99/00316</b>	International filing date (day/month/year) <b>20/05/1999</b>	Priority date (day/month/year) <b>20/05/1998</b>
International Patent Classification (IPC) or national classification and IPC <b>A61K31/70</b>		
Applicant <b>FACULTEIT GENEESKUNDE et al.</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand  <b>16/12/1999</b>	Date of completion of this report  <b>22.08.2000</b>
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer  <b>Greif, G</b>  Telephone No. +49 89 2399 8659



# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL99/00316

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

### Description, pages:

3-5 as originally filed

1,2,2a as received on 26/06/2000 with letter of 23/06/2000

### Claims, No.:

1-6 as received on 26/06/2000 with letter of 23/06/2000

### Drawings, sheets:

1/1 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL99/00316

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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-6
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-6
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-6
	No:	Claims	

**2. Citations and explanations**

**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**



**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. The use of a nucleic acid-binding chemotherapeutic agent for the preparation of a pharmaceutical composition for the treatment of a disease caused by virions, as well as pharmaceutical compositions comprising a nucleic acid-binding chemotherapeutic agent comprising a metal ion complexed therewith, have not been described in the available prior art. Claims 1-6 of the present application therefore meet the requirements of the PCT with respect to novelty and inventive step.
2. Claims 1-6 of the present application fulfill the requirements of the PCT with respect to industrial applicability.

**Re Item VII**

**Certain defects in the international application**

There are discrepancies between the description on p. 4, lines 10-24, and p. 3, lines 18-25. On p. 3, lines 20-21, it is stated that "Fig. 2 shows a graph representing the site of toxicity of bleomycin for macrophages". However, on p. 4, lines 11-12 it is stated that "Figure 1 and Figure 2 show the dose-dependent reduction of the HIV-1 replication".

Therefore, the content and wording of the explanation of the figures 1-4, in the description on p. 4, lines 10-24, is unclear and renders the understanding of the figures impossible.

Furthermore, the description of Figure 4 on p. 4, lines 14-16, "the insignificant effect of bleomycin on the proliferation of lymphocytes is shown in Figure 4", does not match what is indicated in Figure 4, where doses of 1 µg/ml and greater show a significant effect.

Use of a nucleic acid-binding chemotherapeutic agent, and a pharmaceutical composition

The present invention relates to a use of a nucleic acid-binding chemotherapeutic agent, wherein the nucleic acid-binding chemotherapeutic agent is capable of complexing a metal ion, yielding a complex that promotes the formation of hydroxyl radicals from hydrogen peroxide.

Such a nucleic acid-binding chemotherapeutic agent is already known in the art. For example, certain neoplastic tissues (tumours) may be treated with bleomycin. Bleomycin is capable of binding bivalent iron, while the ferro-ion retains its ability to promote the formation of hydroxyl radicals from hydrogen peroxide.

It is the object of the present invention to provide a novel use of a nucleic acid-binding chemotherapeutic agent such as defined above.

According to the present invention the nucleic acid-binding chemotherapeutic agent can be used for the preparation of a virion number-reducing composition.

Surprisingly it has been found that by applying the above-defined nucleic acid-binding chemotherapeutic agent, the virus replication may be inhibited, without visible detriment to the host cell. Without being bound to any theory, applicant believes that the inhibition is specific because the formation of hydroxyl radicals from hydrogen peroxide is promoted especially in virus-infected cells.

According to a preferred embodiment, the nucleic acid-binding chemotherapeutic agent is selected from the group comprising bleomycin, adriamycin, and their derivatives.

These compounds possess excellent metal ion-complexing properties. In particular, they are capable of binding ferro-ions in the body of a patient. This enables the ferroleomycin complex that is formed to promote the formation of hydroxyl radicals from hydrogen peroxide.

Preferably the nucleic acid-binding chemotherapeutic agent is used for the preparation of an RNA virus

replication-inhibiting agent, in particular the nucleic acid-binding chemotherapeutic agent is used for the preparation of a HIV replication-inhibiting agent.

Carter, B.J. et al. (Proc. Natl. Acad. Sci. USA, volume 87, pp. 9373-9377 (1990)) describe the effect of Fe(II)-bleomycin complex on mRNA which codes for reverse transcriptase of HIV-1. The experiment described was performed in a cell-free system. There is no indication that the formation of hydroxyl radicals from hydrogen peroxide is promoted preferentially in infected cells.

The invention further relates to a pharmaceutical combination composition comprising a nucleic acid-binding chemotherapeutic agent comprising a metal ion complexed therewith, which complex is able to promote the formation of hydroxyl radicals from hydrogen peroxide, together with a pharmaceutically acceptable carrier or excipient, and which also comprises an iron-chelating compound which binds iron in a form in which it is unable to promote the formation of hydroxyl radicals from hydrogen peroxide.

Such an iron-chelator combination which optionally comprises two separate pharmaceutical compositions, each of which possessing one of the respective active components, facilitates more specific localization of the formation of the hydroxyl radicals. By using an iron-chelating compound that is unable to penetrate the cells, it is possible to preferentially prevent the formation of ferro-bleomycin complex outside the cells, and consequently also to reduce the damage that such a complex causes. At the same time, the use of an iron-chelating compound that is able to penetrate the cells, will limit the amount of ferro-ions that limit the formation of hydroxyl radicals. In this way at least part of the activation process of the transcription factor *Nuclear Factor kappa B* (NF $\kappa$ B), that can stimulate virus replication may be limited in the cytoplasm. However, it is necessary to ensure that iron is available for bleomycin. A physician may achieve this by choosing suitable doses of both active components, depending on the body weight of the person to be treated, and the person's available iron level. According to a favour-

CLAIMS

1. A use of a nucleic acid-binding chemotherapeutic agent for the preparation of a viron number-reducing composition, wherein the nucleic acid-binding chemotherapeutic agent is capable of complexing a metal ion, yielding a  
5 complex that promotes the formation of hydroxyl radicals from hydrogen peroxide.

2. A use according to claim 1, **characterized** in that the nucleic acid-binding chemotherapeutic agent is selected from the group comprising bleomycin, adriamycin,  
10 and their derivatives.

3. A use according to claim 1 or 2, **characterized** in that the nucleic acid-binding chemotherapeutic agent is used for the preparation of an RNA virus number-reducing composition.

15 4. A use according to claim 3, **characterized** in that the nucleic acid-binding chemotherapeutic agent is used for the preparation of a HIV replication-inhibiting composition.

5. A pharmaceutical combination composition comprising a nucleic acid-binding chemotherapeutic agent comprising a metal ion complexed therewith, which complex is able to promote the formation of hydroxyl radicals from hydrogen peroxide, together with a pharmaceutically acceptable carrier or excipient, and which also comprises  
20 an iron-chelating compound which binds iron in a form in which it is unable to promote the formation of hydroxyl radicals from hydrogen peroxide.

6. A pharmaceutical combination composition according to claim 5, **characterized** in that iron-chelating compound has an iron-chelating capacity which is preferably  
30 at least three times lower, more preferably at least ten times lower than that of the nucleic acid-binding chemotherapeutic agent.